

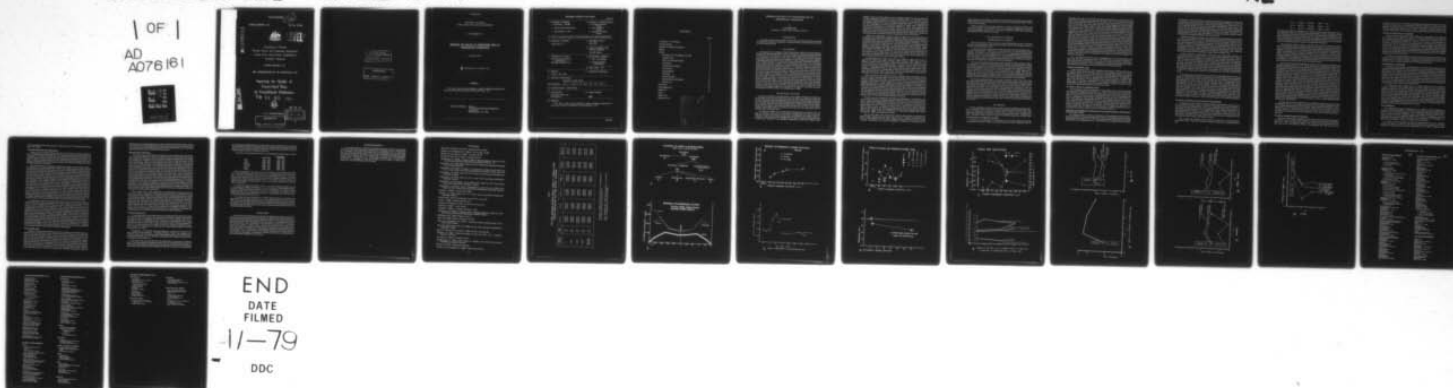
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Armed Forces Food Science Establishment  
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Improving the Quality of  
Freeze-Dried Meat  
by Formaldehyde Stabilization

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J. R. CASLEY-SMITH

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6 IMPROVING THE QUALITY OF FREEZE-DRIED MEAT BY  
FORMALDEHYDE STABILIZATION

10 J. R. CASLEY-SMITH

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SUMMARY

This report reviews results obtained in studies investigating techniques for improving the quality of large pieces of freeze-dried meat. (U)

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# IMPROVING THE QUALITY OF FREEZE-DRIED MEAT BY FORMALDEHYDE STABILIZATION

by

J. R. Casley-Smith

University of Adelaide, South Australia, Australia.

## INTRODUCTION

This report reviews results obtained in studies concerned with the devising of a method for improving the quality of large pieces of freeze-dried meat, optimising this, and ensuring that the procedure was safe.

## THE PROBLEM

This was set out at a meeting in Canberra (Hutchinson, 1970), which was attended by various people who had responded to a circular addressed to Universities and other Research Institutions by the then Director of the Armed Forces Food Science Establishment, Dr. R. C. Hutchinson. He stated (p. 4) that while it was possible to produce very acceptable small pieces of dried meat (and fruit and vegetables), it was impossible to produce consistently acceptable large pieces of dried meat, which could always be reconstituted satisfactorily. Such a product would be of great value for lightweight ration packs for the Australian Forces, being particularly applicable to Australia since Australians are very fond of eating large pieces of meat with a chewy juicy texture. Dr. Hutchinson stated that freeze-drying (reviewed Bengtsson and Bengtsson, 1968; Dowman, 1971; Row, 1971; Venkata-Raman, 1971) was the most successful process so far used in attempts to produce such an article, but that it suffered from a number of defects: "The absorbed water (on reconstitution) does not exactly replace that evaporated as the reconstituted product tends to remain wet on the surface, and dry within, and water can be partly removed by slight pressure. The satiety value is changed (lowered). If the product is stored prior to reconstitution, it usually becomes progressively tough." While these defects would not matter very much if the ration packs were only to be used for one or two days, it is considered that they must be suitable for five to seven days. There is the added technical difficulty that the product should be precooked to some extent and should be freeze-dried (to lessen the weight and to help preserve it). Both these processes are notorious for the way that they reduce the water-holding-capacity (WHC) of meat, i.e. its juiciness (Hamm, 1960).

## THE POTENTIAL SOLUTION

During the conference mentioned above, it was suggested (Casley-Smith, 1970) that the main fault in the freeze-drying technique might well lie in the fact that the freezing rates for such large pieces of meat are necessarily rather slow, which will allow large ice crystals to form, and injure the cellular membranes, especially those forming the cell plasma membranes (the cell walls). This was later confirmed (Casley-Smith, 1973). Hence, once the reconstituting water was added, it could pass throughout the whole of the tissue, and the cellular contents would readily pass to the exterior of the cells. Thus the meat would come to resemble a piece of sponge in soup.

The underlying reasons for the difficulties with freeze-drying are unknown. They are likely to remain so until we have a much better understanding of the basic physics and chemistry of water in the tissues and how these are affected by this process, and by reconstitution. These difficulties are highlighted by the fact that so much water is contained in meat (Fig. 1). However, it was considered (Casley-Smith, 1970) that it might be quite



possible to find an *ad hoc* solution to the immediate problems by applying techniques of tissue stabilisation developed for electron microscopy. Here, it is imperative that the fine structure, and frequently the "fine physics and chemistry", of the tissue be preserved, with special emphasis on the cellular membranes (Sjöstrand, 1967). It was suggested that the lack of stabilisation of these membranes might be the factor chiefly responsible for the difficulties experienced in freeze-drying (Casley-Smith, 1970). These suggestions have indeed been confirmed during this project. Electron microscopy has revealed that such ruptures do occur and that they are largely prevented by the appropriate pretreatment (Casley-Smith, 1973, 1975; Casley-Smith and Ehmann, 1973). Previous work showed that such a pretreatment considerably improves the acceptability of the final product.

Briefly, the pretreatment consists of fixing the tissues with low concentrations of formaldehyde and removing the unreacted reagent by washing in water which also includes phosphate buffers (pH 11) to increase the juiciness and water-holding-capacity (WHC) of the product. When preparing specimens for frozen-section electron microscopy, it is well known that such fixation renders the cells much less susceptible to ice-crystal damage, and probably more natural in appearance on reconstitution after drying following freezing (Sjöstrand, 1967; Bernhard and Leduc, 1967; Bernhard and Viron, 1971; Tokuyasu, 1973). The fixatives bind together the proteins of the cells, especially their membranes, with covalent bonds. It is thus much harder for ice-crystals to rupture cellular membranes and the osmotically-active constituents of the various compartments are much less likely to leave them on reconstitution. Hence water will tend to re-enter these compartments to extents roughly equivalent to the amounts present originally.

Formaldehyde has been used in our work for a number of reasons. It is known to penetrate the tissues very rapidly (Dempster, 1960; Hopwood, 1967, 1969). It is very effective in cross linking the proteins, producing the finest pores in the membranes of any of the conventional fixatives (Sjöstrand, 1967). These pores are quite distinct from those between the cells, upon whose width depends the rapidity of water re-entry during reconstitution — formaldehyde does not alter these intercellular pores (Casley-Smith, 1975). Formaldehyde does have the disadvantage of making the material tougher, but it has been shown that this can be kept to minor levels, which are not significant compared with its advantages (Casley-Smith, 1975; Casley-Smith et al., 1974).

There are, however, some difficulties with formaldehyde; one of the greatest of these is that it is used to produce leather! In order that our steaks might not suffer this fate, it has been necessary to control both the concentration and the time of the formaldehyde treatment. The tendency towards toughness was further offset by treating the meat with phosphate buffers (pH 11) whilst washing out the excess, unreacted, formaldehyde. This greatly increased both the water-holding-capacity (WHC) and rehydration ratio (RR) of the meat. Since too much phosphate was found to induce a soapy taste, it was also necessary to control the concentration of this. In general (Casley-Smith et al., 1974) it was found that the final treatment improved the WHC, RR and juiciness of the meat, and also its texture and tenderness. Flavour was adversely affected, but this was not considered important since it could easily be corrected with additives, while texture and juiciness were the real problems which the treatment had been developed to overcome.

This was, even so, one potentially most significant disadvantage — formaldehyde might act as a poison. Certainly it gained an unsavoury reputation when it was used as a preservative by unscrupulous sausage manufacturers early in this century, but this was when it was simply used in high concentrations as a preservative, with no attempt to remove the unreacted reagent. In fact, it has been fed daily to sheep in relatively far greater doses than likely to be received by men eating our product, for periods of years, with no ill effects (Durand, 1971). Further any administered formaldehyde is rapidly metabolised (Durand, 1971; Neely, 1964), while our formaldehyde is irreversibly combined with protein (Middlebrook, 1949) and metabolised with them.

Also, we have shown that meat normally contains a small amount of formaldehyde, which actually exceeds the levels which remain after our treatment (Casley-Smith and Ehmann, 1973; Casley-Smith et al., 1974). Washing, cooking and freeze-drying remove the unreacted formaldehyde. The reacted formaldehyde is attached to the proteins, and is

digested and used in the body just like any normal food without ill-effects, being converted almost entirely to water and carbon dioxide in a short time (Durand, 1971; Warner, 1972 — personal communication).

However, sheep are not men. Since the gut of a ruminant is indeed so different from that of an omnivore, it was decided to carry out a long-term feeding trial in rats before undertaking extensive testing in men. It has shown (Casley-Smith, 1977) that the rats were quite unharmed after 12 months of this diet.

### *THE ESTIMATION OF RESIDUAL FORMALDEHYDE IN TISSUES*

Since it was crucial to know how much residual formaldehyde was left in the tissues after our various techniques, it was necessary to ensure that we had a reliable method for its estimation (Casley-Smith, 1972).

A technique using the traditional semicarbazide reaction was first used (Wells, 1971 — personal communication). Unfortunately it was found that an unidentified and variable substance was present in the samples which had a very substantial spectrophotometric absorbance near the peak absorbance (252 nm) of the semicarbazone. Many techniques were tried in attempts to remove this substance, or to nullify its effects, but they were unsuccessful in that anything which did this also severely interfered with the detection of the semicarbazone. The semicarbazide reaction was therefore abandoned.

A new test for formaldehyde has recently been described by Bailey and Rankin (1971). This is based on the effect of formaldehyde on the oxidation of p-phenylenediamine (PPD) by hydrogen peroxide; it both greatly accelerates the reaction and produces a different product (Bandrowski's base). The test is much more selective than the previous methods and is as sensitive as all except the J-acid method (Sawicki et al., 1963) which was impractical for our work. The method is very insensitive to many possible interfering substances, and is very selective for formaldehyde. It has the added advantage that the peak spectrophotometric absorbance is 485 nm, which is far removed from that of the interfering material. (In fact the meat extracts have quite a low absorbance at this wavelength).

There is one unfortunate defect in the Bailey and Rankin method. This is that, unless one uses a substantial excess (20 times) of the PPD, the reaction does not follow the simple Beer-Lambert law. Thus the range of the test is relatively limited (0.5 to 2.5  $\mu\text{g/ml}$ ). Since the residual formaldehyde in our material was found to vary over a range of  $\sim 100:1$ , this 5:1 range would have meant many unsuccessful trial dilutions and much waste of time and samples. It was therefore decided to plot the standard curve for the absorbance at many concentrations of formaldehyde and use a non-linear statistical technique to estimate the Standard Deviations and Standard Errors of many points along the curve. The use of this technique is not very much more complex than the use of the standard technique. It is, however, essential to standardise the amount of PPD very carefully. Thus it was found that using a gravimetrically-checked syringe rather than a normal pipette decreased the Standard Errors by 2/3.

### *THE METHOD*

Initially, whole sheep and goats were perfused with the formaldehyde solution, followed by the washing fluid to remove the unreacted formaldehyde (Casley-Smith, 1972). However, it was found that immersing 2 cm thick pieces of steak in the fluid was just as satisfactory (Casley-Smith and Ehmann, 1973), and certainly much easier — especially with cows — although it would not be of any use with much thicker pieces, such as whole roasts.

#### **The Penetration of Formaldehyde into Steak**

The amount of penetration of formaldehyde into 2 cm thick steaks was estimated (Casley-Smith and Ehmann, 1973) by immersing pieces into 0.4% formaldehyde (in 0.1 M

phosphate buffer, pH 7) for 8 hours, and taking samples of 0.2 cm intervals through the meat. (Here, as elsewhere unless specified, the steaks were from two-year-old cow psoas muscle). The results are shown in Fig. 2, where it can be seen that there is quite a good, high, concentration, even in the centre of the meat. This corresponds well with the observations, on very much thinner pieces of tissue, by Dempster (1960) and Hopwood (1967). They found that the "fixed" region of tissue had a depth (D) which was related to the time of immersion (T) by the formula:  $T = kD^e$ , where k and e are constants relating to the type of fixative, its concentration, and the nature of the tissue. For 4% formaldehyde, Dempster (1960) found that k was 1.14 and e was 1.25, where D was in mm and T in hours, giving much more rapid fixation than most of the other fixatives he tried. At 9 hours, formaldehyde had fixed 5 mm depth of tissue, and its rate of penetration was increasing compared with those of the others. Of course, the concentration we used (0.4%) was considerably less than that used by Dempster (4%), but we only required a certain minimum stabilization, and not the complete fixation desired and measured by Dempster. Hence 8 hours immersion in 0.4% formaldehyde is likely to give adequate penetration into steaks 2 cm thick. Fig. 2 shows that the immersion method is not likely to be successful for pieces of meat much thicker than 2 cm since the measured residual formaldehyde in the centre of the steak is not significantly different to the naturally occurring formaldehyde. The difference between expected and observed residual formaldehyde levels (Fig. 2) in the unwashed steak near the surface is most likely due to the evaporation of formaldehyde during frozen storage of the steaks prior to assaying.

Fig. 2 also shows the amount of free formaldehyde left in the various depths of the tissue after 24 hours of washing in water. It can be seen that the amounts are reduced to below the normally occurring levels in the centre of the meat, while they are most remarkably reduced at its periphery. This is in keeping with our observations (Casley-Smith, 1972) that washing the meat can remove even the naturally occurred formaldehydes.

#### Residual Formaldehyde Levels

It was found (Casley-Smith, 1972; Casley-Smith and Ehmann, 1973) that 8 hours was the optimum period for the immersion of meat in the formaldehyde solution. Fig. 3 shows the residual amount of this left after 24 hours of washing (Casley-Smith and Ehmann, 1973). It can be seen that there is normally quite a high concentration of formaldehyde in the muscle 1400  $\mu\text{g/gm}$ . (Such is the specificity of the test used that it is indeed almost certain that this can only be formaldehyde). This is greatly reduced by washing to 400  $\mu\text{g/gm}$ . Even the maximum concentration of formaldehyde (0.8g %) only yielded a final concentration of 700  $\mu\text{g/gm}$ . Hence it can be seen that such a treatment would not increase the residual formaldehyde concentrations, rather the reverse.

#### Formation of Natural Formaldehyde in Meat

The formation of natural formaldehyde in meat, according to the time which elapses after death was investigated (Casley-Smith et al., 1974). The naturally occurring formaldehyde levels were determined 2 hours after death, and each day for 5 days. The results are shown in Fig. 4. It can be seen that, as noted before, there is quite an appreciable initial level of formaldehyde. This increases considerably after 3 days, with a slight fall after 5 days. The biochemistry underlying this increase in formaldehyde is obscure. It must be due to the release of bound formaldehyde or its production during autolysis. Which of these occurs, and what the precursors are, is unknown, (although Harada and Yamada [1971] report the progressive formation of formaldehyde from trimethylamine in some fish). It is virtually certain that the substance we are measuring is indeed formaldehyde since, as mentioned in the previous reports, the test we use has great specificity.

#### The Effects of Formaldehyde Concentrations and Pre-treatment Storage on the Rehydration Ratios and other criteria

The hanging of meat is normally so important for the tenderness of the final product, that it was essential to discover if storage had an effect on formaldehyde-treated meat also (Casley-Smith and Ehmann, 1973). Fig. 5 shows the results of treating meat with various concentrations of formaldehyde after 0, 1.5 and 48 hours of storage at 2°C after the death



of the animal. It can be seen that in the control specimens there appeared to be little free formaldehyde initially, but that this increased during storage (presumably as a result of the autolytic changes mentioned above), with subsequent washing-out as shown by the specimens treated by washing, but with treatment with 0% formaldehyde concentration. It can also be seen that 0.4% formaldehyde concentration is the minimum which sterilizes the meat for all the storage times, although lesser concentrations do so for shorter pre-treatment storage periods. (Naturally such sterilization would be very greatly enhanced in the desiccated condition of the freeze-dried product). It can be seen that the amount of residual formaldehyde is not greatly affected by the storage periods.

Residual formaldehyde, however, is by no means the only consideration. The rehydration ratio is one measure of the likely acceptability of the product. The effect of pre-treatment storage (2°C) on this is shown in Fig. 6 (Casley-Smith and Ehmann, 1973). It can be seen that storage between killing and processing is, if anything, slightly deleterious to the product. The effect of formaldehyde concentration on rehydration ratio is seen in Fig. 7. Untreated meat (control) had a significantly lower rehydration ratio than (0.0, 0.1, 0.3%) formaldehyde treatment. However higher formaldehyde concentrations (0.4, 0.8%) had significantly lower rehydration ratios than the (0.0, 0.1, 0.3%) group. Hence treatment concentrations above 0.3% formaldehyde adversely affect rehydration ratio.

Various parameters of acceptability (rehydration ratio, texturometer hardness, and cooking weight loss) are shown in Fig. 7 for reconstituted freeze-dried steak. They are compared for various formaldehyde concentrations, some samples having been frozen to -12°C immediately after treatment and washing (full black symbols), the rest having been stored at +2°C (non-filled symbols) until all samples were freeze-dried. No statistically significant differences were observed between these samples held at -12°C after treatment and those held at 2°C. Increasing concentrations of formaldehyde increased the hardness of the product as measured in the texturometer. It can be seen from these results that 0.3-0.4% formaldehyde is about the optimum under these conditions, and it appears quite acceptable in Figs. 3, 5, 6 provided that treatment is commenced within 1.5 hours of death of the animal.

It can also be seen from these results that there is no advantage, rather the reverse, in storing the meat between killing and pre-treatment. This might perhaps have been expected when one considers what hanging normally accomplishes, and the effects of formaldehyde. Hanging allows the meat to pass into and through rigor, which is brought about by the decomposition of the ATP, causing muscular contraction (Price and Schweigert, 1971). Formaldehyde, however, will fix the muscles in relaxation (assuming they are relaxed at death, or just after it). Thus the meat will be likely to be much tenderer than would be the case with untreated meat. It may well be the reason why such treated meat is still quite tender (*vide infra*), in spite of formaldehyde being used to produce leather at much greater concentrations and periods of immersion. Immediate commencement of fixation is also important in order to minimise the post mortem disintegration of the cells which commences so rapidly after death. (It should be mentioned in this regard, however, that some experiments indicate that about 1 hour's delay after death is not of great consequence.)

#### **The Effects of Alterations in pH on the product using Phosphates**

It is apparent that the formaldehyde-treated product, while usually an improvement on the untreated freeze-dried product, is still not as acceptable as the control samples on nonfreeze-dried meat—although there is often little difference between the two. Since it is well known that raising the pH of meat considerably causes a great improvement in its water-holding-capacity (WHC) — Hamm (1960) — we decided to adjust the pH of the washing water by using phosphate buffers in order to alter the pH of the meat before it was freeze-dried in the hope that the pH would remain unaltered upon its reconstitution and thus improve its WHC, juiciness, tenderness and texture.

Three different pH phosphate buffered washing solutions were tried (Casley-Smith and Ehmann, 1973). These were made up as follows in proportion by weight (anhydrous salt).

pH 11	$\text{Na}_4\text{P}_2\text{O}_7 + \text{Na}_3\text{PO}_4$	(100.1 + 1.0)
pH 9	$\text{Na}_4\text{P}_2\text{O}_7 + \text{NaH}_2\text{PO}_4$	(100.0 + 13.7)
pH 7	$\text{Na}_4\text{P}_2\text{O}_7 + \text{NaH}_2\text{PO}_4$	(100.0 + 82.4)

The final concentration of phosphate salt in each washing solution was carefully held at 2.0% (anhydrous salts) w/v so as to minimise differences that could be attributed to varying total phosphate concentrations. However the effect of individual species of phosphate could not be controlled as their concentration had to be varied to achieve the desired pH. All slices were washed by immersion in their allotted phosphate solutions for 12 hours. Some meat was freeze-dried; other samples were not. Figs. 8-13 show the effect of pH of washing water on various characteristics in the case of treated freeze-dried steak.

The taste panel results are shown in Figs. 10-13. The controls in each taste character more or less reflect the generally observed differences between freeze-dried meat and fresh meat, with little difference in juiciness (usually a greater difference is observed, this result is probably a reflection on the cut of meat used), a significant reduction in tenderness and overall texture ratings, and a somewhat depressed but not significantly different flavour rating. The plotted ratings also reflect the effect of cooking method on nonfreeze-dried phosphate washed meat slices. Grilling lowers juiciness but improves flavour at all levels of pH treatment. For the purposes of comparison the braised nonfreeze-dried samples should be compared with the lightly cooked freeze-dried samples first (but taking into account the grilled samples) and then compared to the control sample results.

It can be seen that juiciness is significantly reduced by the freeze-drying process despite phosphate washing except at pH 11, where juiciness is not significantly different from controls (both freeze-dried and not freeze-dried) and nonfreeze-dried treated + washed meat. The significant increase in the tenderness rating of phosphate washed (pH 11) nonfreeze-dried meat (Fig. 11) above untreated nonfreeze-dried meat washed at pH 11 shows that the improved tenderness is a result of treatment with formaldehyde and phosphate (pH 11) washing and that this desirable quality is not lost during freeze-drying. In fact the pH 11 washed sample shows a marked improvement over normal untreated freeze-dried meat. However at pH 7 and 9 phosphate washing, freeze-drying significantly reduced tenderness. Similarly, the overall texture, which was appreciably reduced by freeze-drying, was considerably increased by raising the pH to 11 and exceeded significantly the overall texture rating for normal freeze-dried meat. The flavour, however, was adversely affected by the phosphates used to raise the pH, which accounted for a reduction in the score for flavour in the treated meat, compared with both the fresh meat and the meat which was simply freeze-dried. It is significant that there was no apparent change in flavour rating at various pH levels. This effect can almost certainly be overcome by the use of less phosphate (see below), since the amount used was considerably in excess of that needed simply to maintain the appropriate pH. The amount of phosphate, however, was within the limits set by the American Food and Drug Administration which specifies a maximum limit of 0.5% phosphate in meat products. Our washing solution at 2% was calculated to result in a final product phosphate concentration of below 0.5%.

It can be seen from Fig. 8 and 9 that the more objective, although less conclusive tests of rehydration ratio for all the raised pH samples and cooked weight loss especially for the pH 11 samples also indicate that controlling the pH has very significant advantages. It would appear that, with the reduced amounts of buffer, raising the pH of the meat by means of the wash, before it is freeze-dried may well prove to be a quite significant method for improving the acceptability of the product especially if the pH of the reconstituting water is also raised.

#### **The effect of reduced Phosphate Concentration**

Previously treated samples, assessed by the taste panel, had indicated that 2% phosphate concentration in the washing solution resulted in a "soapy taste". An experiment (Casley-Smith et al., 1974) was designed to determine the effects of reducing this concentration to half, both to see if the beneficial effects of the phosphate were retained (e.g. on the

rehydration ratio) and if the reduced concentrations eliminated the soapy taste. In non-freeze-dried samples there is no significant difference between the control and treated samples with respect to flavour. However the panel noted that the meat was not of a characteristic meat flavour, tending to be "bland". Significantly increased tenderness and texture ratings resulted from the higher pH washings when compared to the control ratings.

#### **The effect of Freeze-drying on Treated Meat**

Cooking (before freeze-drying) removed ~40% of the initial weight for the control and pH 7 samples, but only ~30% of the pH 9 and 11 samples (Casley-Smith et al., 1974). The texturometer-assessed hardness was higher than the controls in the nonfreeze-dried pH 7 group, but not significantly different in the pH 9 and 11 groups. It was increased in all the treated groups after freeze-drying and reconstitution. In the material to be cooked after reconstitution, the pH 11 group had considerably increased rehydration ratios compared with the other treated groups and with the controls. They lost slightly less water during cooking. The slightly harder texturometer assessment is not statistically significant. Cooking was found to considerably reduce the residual formaldehyde levels. All taste panel-assessed qualities were considerably improved in the nonfreeze-dried samples by the pH 11 treatment, compared with the pH 7 and 9 ones.

The taste panel results indicate that freeze-drying reduced juiciness and flavour in the treated meat, with insignificant change in overall texture and tenderness. In the freeze-dried material, the pH 11 treatment showed a considerable improvement over the controls in the rehydration ratio. The other differences were not statistically significant.

#### **Storage Stability of Treated Meat**

Accelerated storage stability data (Casley-Smith et al., 1974) indicate that there is no significant difference between the control tested soon after freeze-drying and the accelerated storage control with respect to texture and flavour. Accelerated storage did not significantly affect flavour in any treatment and no taste panel comments were noted about off flavours. However texture ratings were somewhat lower. This is probably due to water penetration problems which were experienced during rehydration, and which can be seen from the rehydration ratio figures. The taste panel did comment favourably on the juiciness of the pH 7, 9 and 11 treated samples. In fact a comparison of the taste panel comments towards non-stored and stored treated freeze-dried samples indicated that the stored samples were more acceptable, overall, than the non-stored samples.

The storage trial results (Casley-Smith et al., 1974) indicate there is not significant change in rehydration ratio in either treated or control samples with time. There was a significant increase in texturometer hardness in the control series over the 3-month storage period. The taste panel results after 3 months storage show there was generally a lower overall texture in the treated samples when compared to the controls. Comparing initial ratings and those obtained after three months storage, it is of interest to note the tendency for the control samples to improve their ratings after storage. This is particularly evident with control samples cooked for 10 or 15 minutes in the tenderness rating. The flavour rating is not appreciably different, although there was also an improvement in flavour with storage for control samples cooked for only 0 to 5 minutes. The treated samples after storage indicated a slight overall improvement in flavour.

#### **The Final Methods**

The recommended treatment (Casley-Smith et al., 1974) is a 4 hour exposure to a solution of 0.3 g% formaldehyde in a buffer consisting of 0.274 g%  $\text{Na}_4\text{P}_2\text{O}_7$  and 0.226 g%  $\text{NaH}_2\text{PO}_4$  (pH 7) with 9 g% sucrose. It is then transferred to 0.3 g% formaldehyde for 4 hours in a buffer consisting of 0.495 g%  $\text{Na}_4\text{P}_2\text{O}_7$  and 0.005 g%  $\text{Na}_3\text{PO}_4$  (pH 11) with no added sucrose. This is the combined method, where there is no subsequent wash. The alternative recommended method is 8 hours in the first solution (pH 7), followed by a wash 12 hours in the pH 11 solution, but without the formaldehyde.

The second method removed more residual formaldehyde, but the former, combined, method still resulted in levels less than those naturally occurring. However, the second method gave improved results in general (Casley-Smith et al., 1974). If cooking is used



after treatment but before freeze-drying, the optimum time is 10 minutes at 85°C (Casley-Smith et al., 1974).

#### **Taste Panel Results and other characteristics**

These have been mentioned above (Figs. 10-13). It can be seen that the pretreatment with formaldehyde and phosphate before freeze-drying significantly improves the quality of the product. In particular the WHC, RR, and juiciness are improved compared with the untreated controls. The texture and tenderness is also improved, although not so greatly. The flavour is slightly adversely affected, but it is possible this might be overcome by the use of additives. Storage improves the characteristics rather than the reverse. Hanging, after killing but before treatment is unnecessary.

Both the treated and the untreated meat showed changes from the normal fine structural appearance of muscle (Casley-Smith, 1975): the myofibrils appeared slightly swollen and their fine structural detail was obscured; the cellular membranes were well preserved in the treated sample, although somewhat obscured by a loosely floccular, granular material which seemed to adhere to them from the cytoplasmic matrix—in the untreated samples they were much less well preserved and were usually invisible, added to which the cytoplasmic matrix contained very coarse granules which were presumably derived from the much altered matrix material. Cooking produced its usual destructive changes; these were particularly noticeable in the untreated material, where the cellular compartments in general, all the organelles, and the myofibrils were all very disintegrated. In the treated samples the destruction was much reduced, even the cellular membranes remained largely intact, and the myofibrils were usually separated and remained discrete. Freeze-drying did not alter the last pictures very much except to cause some of the matrix material, both within the cells and between them, to coalesce somewhat on the other structures. Again, reconstitution after freeze-drying did not alter the pictures very much except to apparently cause the material mentioned as being coalesced above, to come back into its previous positions. Storage either with or without reconstitution, caused a certain slight blurring of the pictures. The cellular organelles, and membranes, appeared to be somewhat less discrete than in the unstored material. Frozen sections (Casley-Smith, 1973) have confirmed these findings.

It would appear that as far as the general preservation of the material is concerned, the treatment keeps the cellular organisation more intact, especially after cooking. From the point of view of reconstitution after freeze-drying this is likely to be a good thing, in terms of keeping the osmotically active substances in their normal places and so cause the re-entering water to return to approximately its normal locations. From the point of view of tenderness, however, it would appear that this might be deleterious in that the cooking has effects which are contrary to those of the treatment. (Precooking untreated meat has also been shown to be advantageous—Younger and Baigen, 1965). In fact, however, as mentioned above it has been shown (both objectively and by taste-panel) that the treatment retains, or if anything slightly increases the tenderness. We must conclude, therefore, that cooking has more than one effect on the meat. One of these increases tenderness, the other destroys the cellular organisation. In fact this is reasonable: the effect of cooking on the mucopolysaccharides and collagen fibres in the meat is to destroy and solubilize them — increasing its tenderness; its effect on the cellular membranes is very unlikely to add to the tenderness of the meat since these are such small and relatively fragile objects.

#### **The intercellular gaps**

These were measured using transmission electron microscopy and the Means and their Standard Errors are recorded in Table 1. Since the width of the gaps has a very important effect on the speed with which water can enter the sample (the quantity of water varies with the cube of the width of the gaps, and the mean velocity with the width), it can be seen that the wider the gaps, the more rapidly will the product be reconstituted. However speed of reconstitution has never been a problem with either the untreated or the treated, freeze-dried meat. Hence this consideration is not important. Why freeze-dried meat has no problem with reconstitution can be seen from the fact that it quadruples the normal widths of the gaps, while these are increased to 7-8 times normal after storage; similar, although slightly narrower, gaps are found in the treated meat. Although the treatment might be

expected, a priori, to keep the gaps narrow in fact it allows them to become wider. It widens them initially itself, although tending to maintain their dimensions to some extent after this. We can thus see why one possible problem with the treatment does not occur. These direct measurements also confirm the findings mentioned earlier.

### Scanning Electron Microscopy

The material used for transmission microscopy was also examined with scanning electron microscopy (Casley-Smith, 1975). As mentioned earlier, the gaps between the muscle cells increased with cooking and with freeze-drying, storage and reconstitution after storage, but reconstitution immediately after freeze-drying retained the gap-size almost to that before freeze-drying. Again, the treated samples were found to have very similar gaps to those of the untreated samples. The preparation techniques for SEM did not preserve the plasma membranes of the cells, even in those specimens where the membranes were known, from transmission spectroscopy, to have survived the experimental manipulations. The dehydration of the specimens caused the water to be removed from between the myofibrils, at least in the regions near the surface where we could examine them. This made it easy to examine the surfaces of the individual myofibrils. It could be seen in both the treated and the untreated specimens that the basic striations were still visible; however overlayed on these surfaces were accumulations of material from the cytoplasmic and interstitial matrices. In the treated samples these had a fine, and often filamentous appearance; in the untreated specimens they were much more granular, forming compacted lumps. Similarly, on viewing the edges of the myofibrils, it appeared that they were far less clearly defined and more blurred than in the treated samples. The same appearance was seen at the cut surfaces of the treated versus the untreated samples. Again, cooking seemed to increase these effects, but there were still clearly visible differences between the treated and untreated specimens at all stages of the experimental manipulations.

These results confirm, both qualitatively and quantitatively, those found with the transmission electron microscope. The remarks made earlier about the preservation of the fine structure by the treatment, but its permitting water to penetrate easily and not adding to toughness still apply. These factors are clearly of the utmost importance for the acceptability of the new product. While it is obvious that they have to be determined directly by taste panels and less directly by objective measurements of large samples, the present observations are important in that they show why these effects occur. This is not particularly important for the present treatment of beef, but it may well be the case for treatment of other meats, or if other sorts of treatment are investigated, perhaps by other workers some time in the future. An understanding, even if only partial, of the reasons why these effects occur will facilitate obtaining the optimum conditions elsewhere.

### Long-Term Feeding Trial

As mentioned earlier, much greater amounts of formaldehyde have been fed to sheep than are ever likely to be eaten by men consuming treated food. It has been found that 2 g per sheep per day of formaldehyde does no harm over ~2 years (Durand, 1971); this is equivalent to ~6 g of formaldehyde in a man. In addition, this formaldehyde is by no means all combined with the food, since no effort was made to wash out the uncombined substance. In our food a great effort is made to remove any unreacted formaldehyde, and in fact the levels we finally attain are rather less than those normally present in the meat (Casley-Smith et al., 1974).

Formaldehyde, in sufficiently high concentrations, is a poison (as of course are so many commonly used substances — including foodstuffs). Hence it was necessary to be completely certain that the treatment was safe before exposing troops to it for long periods. This was dictated not only by humanitarian considerations and medical ethics, but by a consideration of national security.

Fifty control rats were used and fifty ate treated meat (1 g per day for 5 days per week). This would equal the formaldehyde content of 5 g of treated steak per day! After 12 months both groups appeared equally active and healthy. At the end of the 12 months the mean weight of the animals eating untreated meat was 300.1 (5.14) g; that of those eating

the treated meat was 294.2 (5.61) g. (The Standard Errors of the Means are given in brackets after the Means). There are no significant differences between these two groups. The growth gains over the 12 months were 149 (7.3) g for the untreated group and 145 (5.8) g for the treated one; again there was no significant difference.

The results of the biochemical estimates of formaldehyde are shown below (in  $\mu\text{g/g}$ ):

	Normal Meat	Formaldehyde Treated Meat
Brain	823 (62)	934 (79)
Liver	1310 (82)	1260 (94)
Kidney	1270 (75)	1300 (83)
Stomach	1050 (67)	1110 (75)
Ileum	981 (74)	1300 (69)
Muscle	1000 (50)	950 (76)

With the exception of the ileum, where the difference is very significant ( $P < 0.001$ ), none of the other differences are significant. Since the specimens were not washed in any way — except for a very brief rinse to remove the gross contents of the gut — it is highly likely that the increase found in the ileum was caused by residual formaldehyde from the ingested meat, rather than being actually present in the lining of the ileum. This did not appear in the stomach, perhaps because the meat was less finely divided here so that the wash removed all of it.

It is clear that eating the treated meat for a year did not cause significant increases in the amounts of residual formaldehyde in the various organs. The histological examinations also revealed no significant differences between the two groups. In all cases the organs appeared normal. Since it was found that there was an increase in the numbers of small round cells in the gut of the sheep (Durand, 1971), a special search was made for signs of chronic inflammation. None were found.

It is evident that the rats suffered no ill effects from this diet. This is similar to the sheep which ate even larger relative amounts of formaldehyde for twice as long (Durand, 1971). Here, even the mild evidence of chronic inflammation of the gut, which was found in the sheep, was not observed. Thus formaldehyde treatment of meat, followed by cooking and freeze-drying, will give a product which can be continuously consumed by men for very long periods without ill effects.

### CONCLUSIONS

It can be seen that pretreatment of meat with formaldehyde does indeed overcome the problems which were mentioned initially (Hutchinson, 1970). It does largely prevent injury to the cellular membranes by the large ice crystals which normally form during freeze-drying. Objective and taste panel findings indicate that the product is a considerable improvement over untreated meat. This is particularly so when the washing is done at pH 11, thus raising the pH of the product. Long-term tests in rats confirm the results of even longer tests in sheep, showing that the product is harmless. Indeed, it appears that meat normally contains a surprising amount of formaldehyde — which is largely removed by the washing included in this treatment. Thus it appears that the formaldehyde pretreatment of frozen-dried steak offers significant advantages. Formaldehyde treatment improved many characteristics of meat not subjected to subsequent freeze-drying.



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TABLE 1

**THE MEAN DISTANCES BETWEEN MUSCLE FIBRES AT VARIOUS STAGES  
DURING PREPARATION, WITH AND WITHOUT TREATMENT**

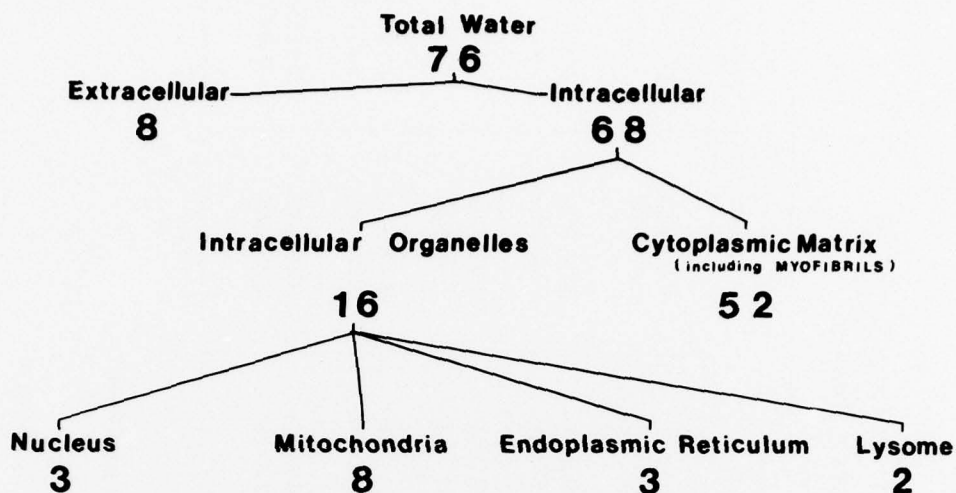
PERIOD OF HANGING	TREAT- MENT	NOTHING	COOKED	COOKED + F.D.	COOKED, F.D., REC.	COOKED, F.D., STORED	COOKED, F.D., STORED, REC.
0	Yes	1.14(0.28)	3.01(0.51)	4.71(0.58)	3.36(9.87)	5.41(1.05)	7.07(1.41)
	No	1.84(0.30)	4.19(0.71)	3.88(0.54)	2.225(0.36)	8.82(1.99)	7.63(1.72)
1 week	Yes	1.88(0.60)	2.11(0.28)	4.96(1.42)	3.16(0.41)	6.59(1.11)	6.62(0.99)
	No	1.00(0.21)	1.90(0.43)	4.32(1.06)	3.09(0.45)	7.42(2.44)	11.0 (1.20)
2 weeks	Yes	3.26(0.73)	0.53(0.10)	5.54(0.85)	1.95(0.54)	7.07(1.20)	8.82(1.00)
	No	0.43(0.12)	3.16(0.65)	5.42(1.31)	2.54(0.52)	7.98(1.53)	10.6 (1.18)
3 weeks	Yes	0.30(0.03)	2.47(0.46)	2.59(0.42)	3.06(0.63)	8.19(1.65)	8.19(1.13)
	No	1.15(0.42)	3.91(0.60)	4.82(0.97)	2.46(0.47)	8.33(1.49)	5.59(1.25)
Means over all periods	Yes	2.15(0.34)	2.03(0.23)	4.45(0.47)	2.88(0.32)	6.82(0.63)	7.68(0.57)
	No	1.29(0.16)	3.29(0.32)	4.61(0.49)	2.59(0.22)	8.16(0.79)	8.69(0.74)

The figures in brackets, (following the Means) are their Standard Errors.  
The number of observations in each group was 10.

F.D. = Freeze-dried; Rec. = Reconstituted; Storage was for 9 months at  
20°C in the freeze-dried condition in sealed packages.

## Location of water in muscle tissue

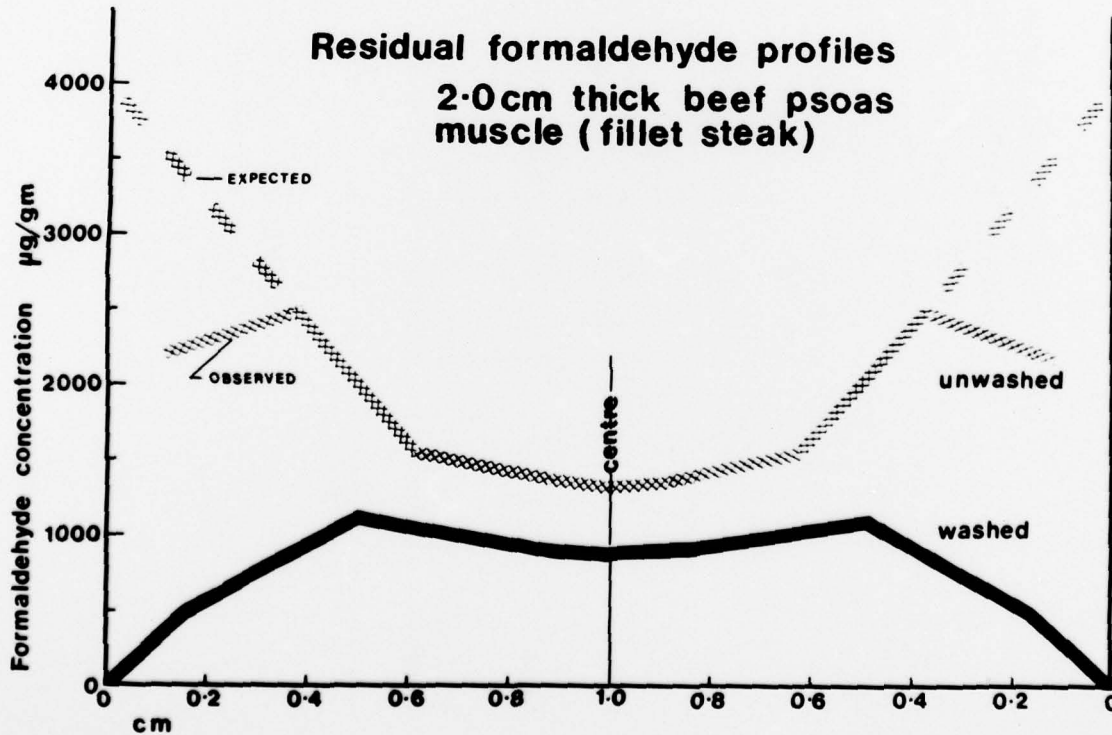
Water as % of total live mass



1

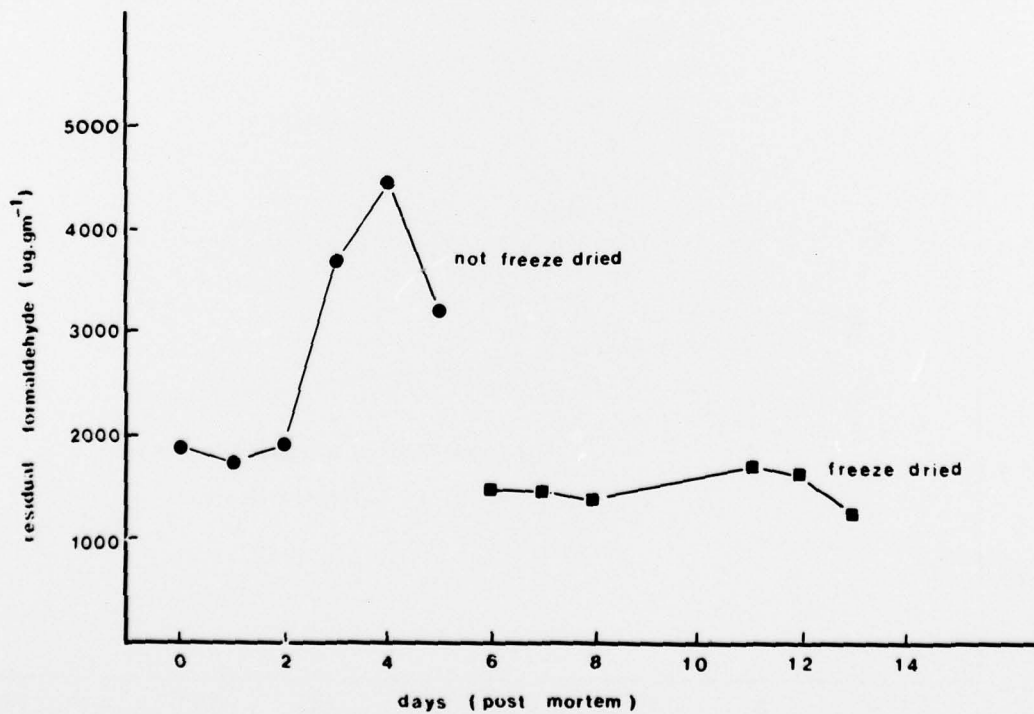
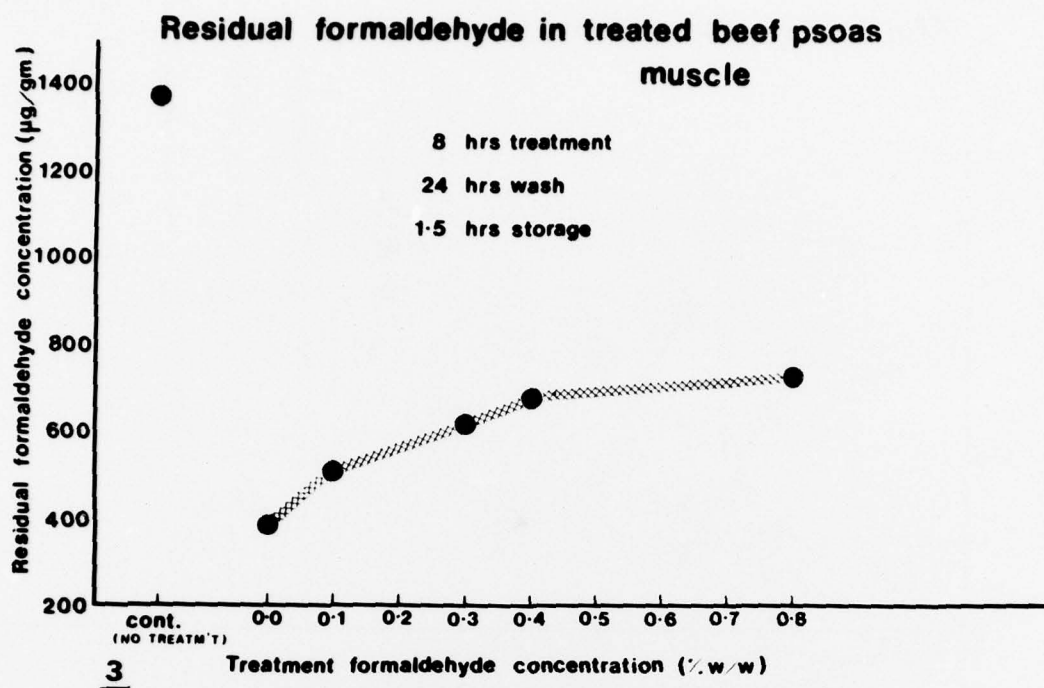
## Residual formaldehyde profiles

2.0cm thick beef psoas muscle (fillet steak)

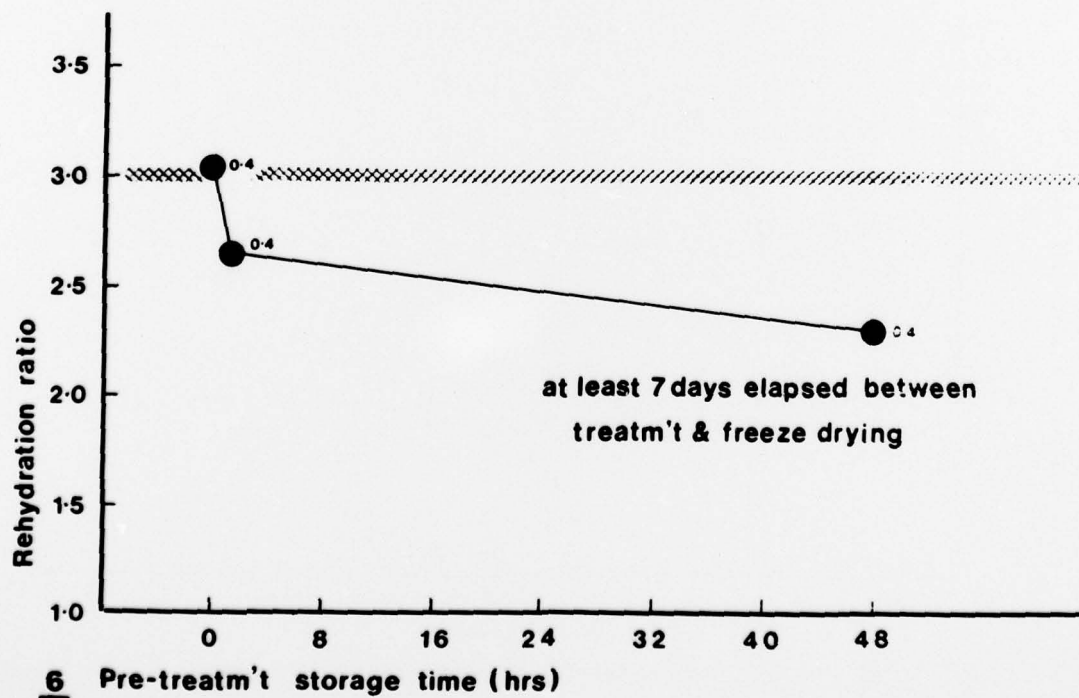
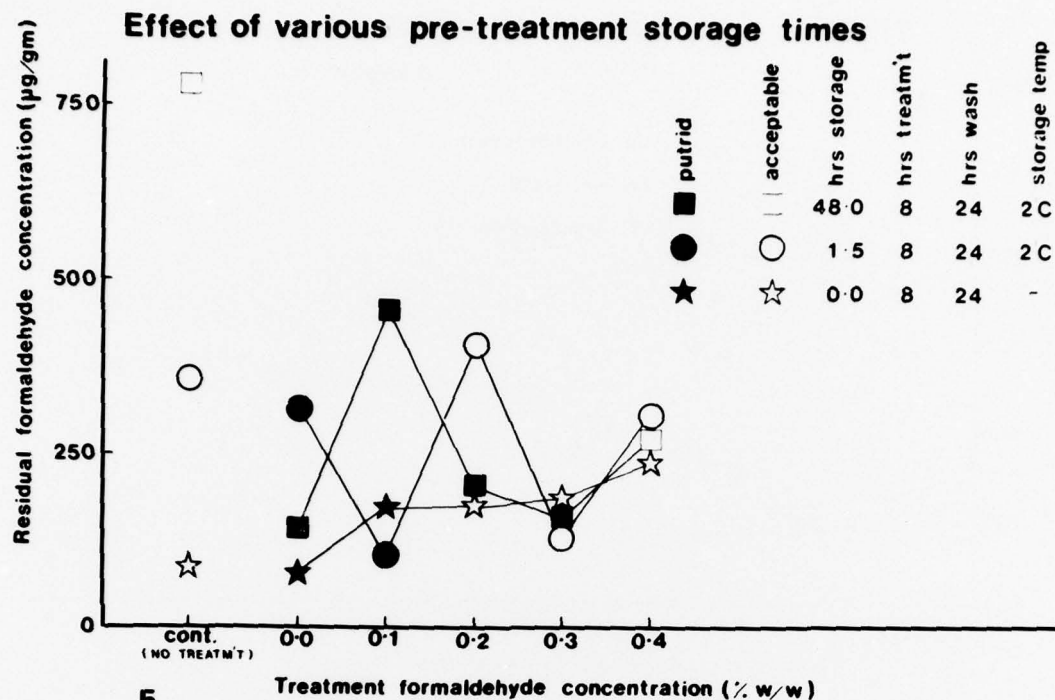


2



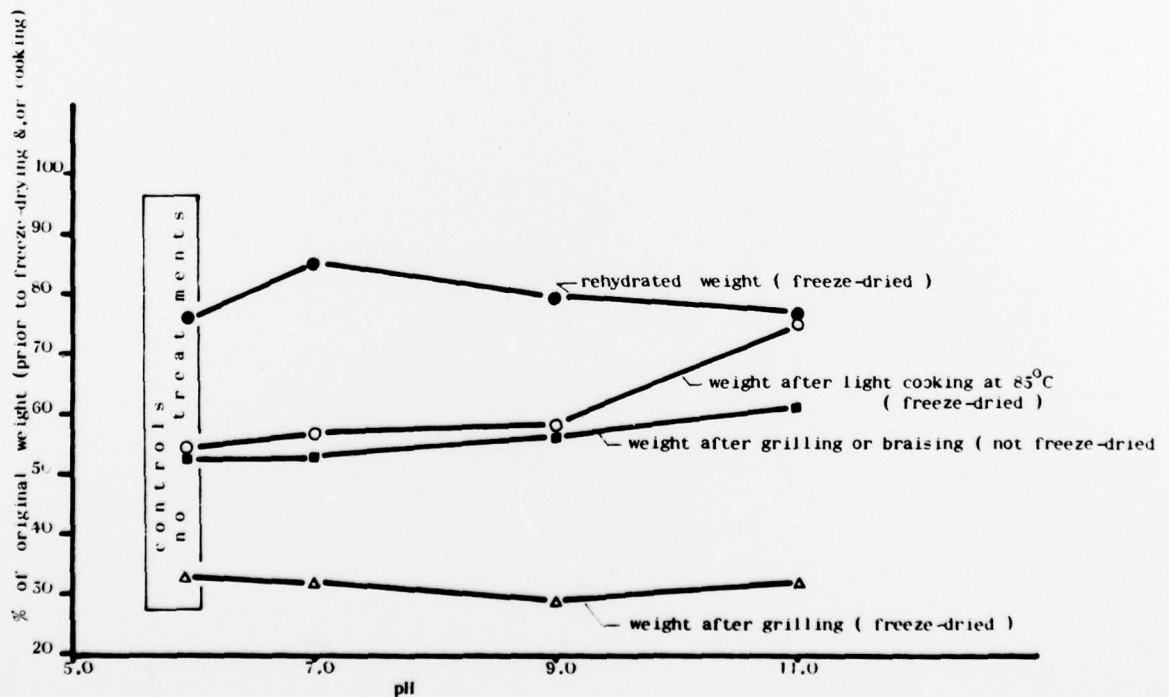
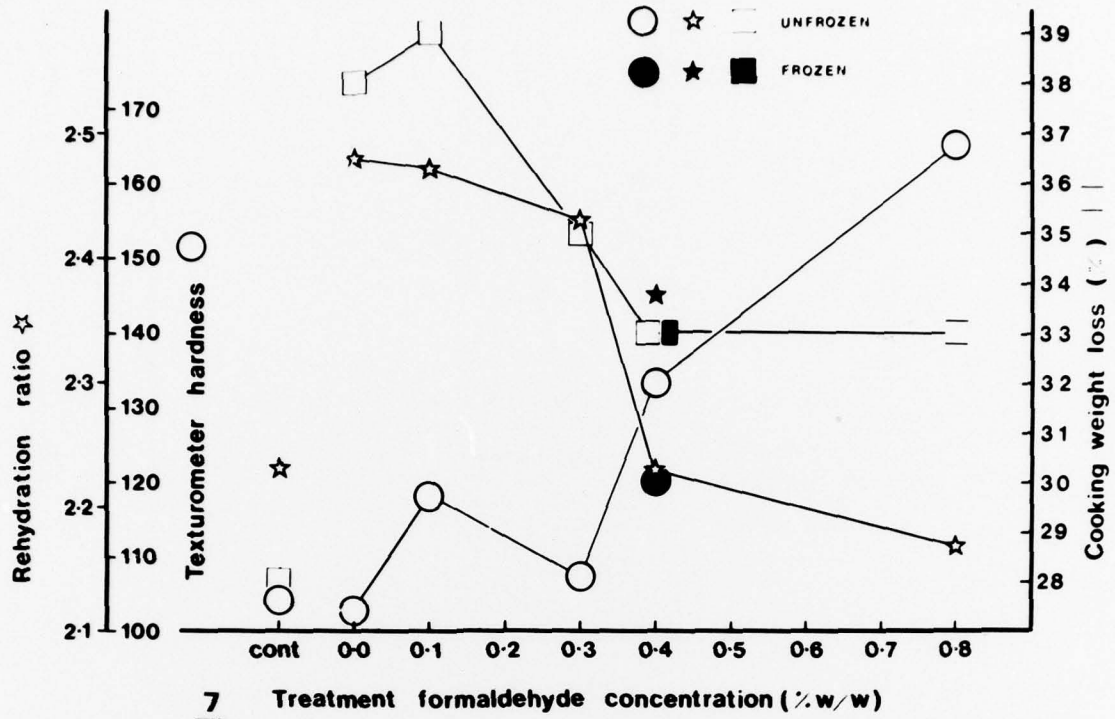


**4** NATURAL FORMALDEHYDE IN POST MORTEM RAW BEEF

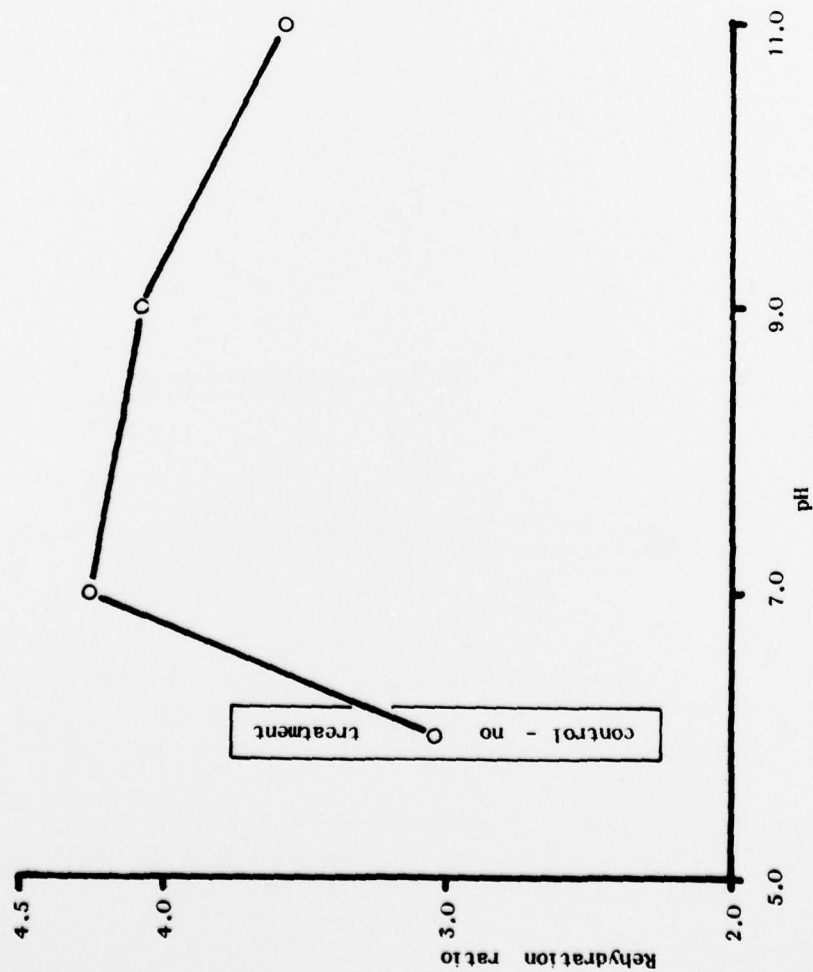




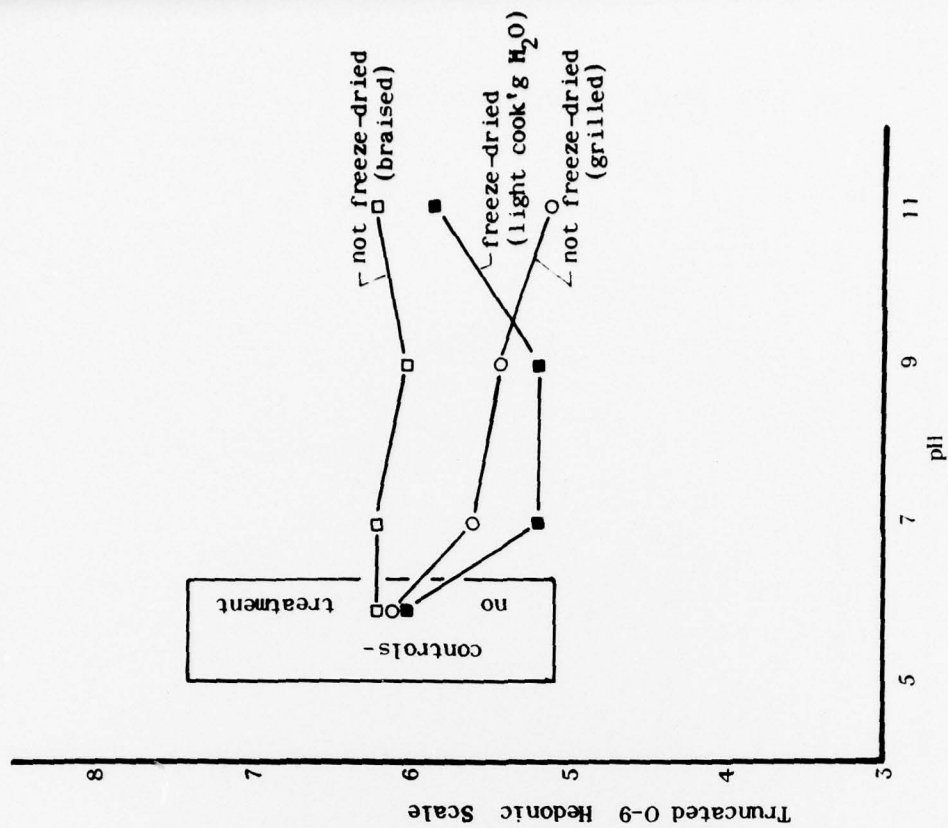
# Treated beef psoas muscle



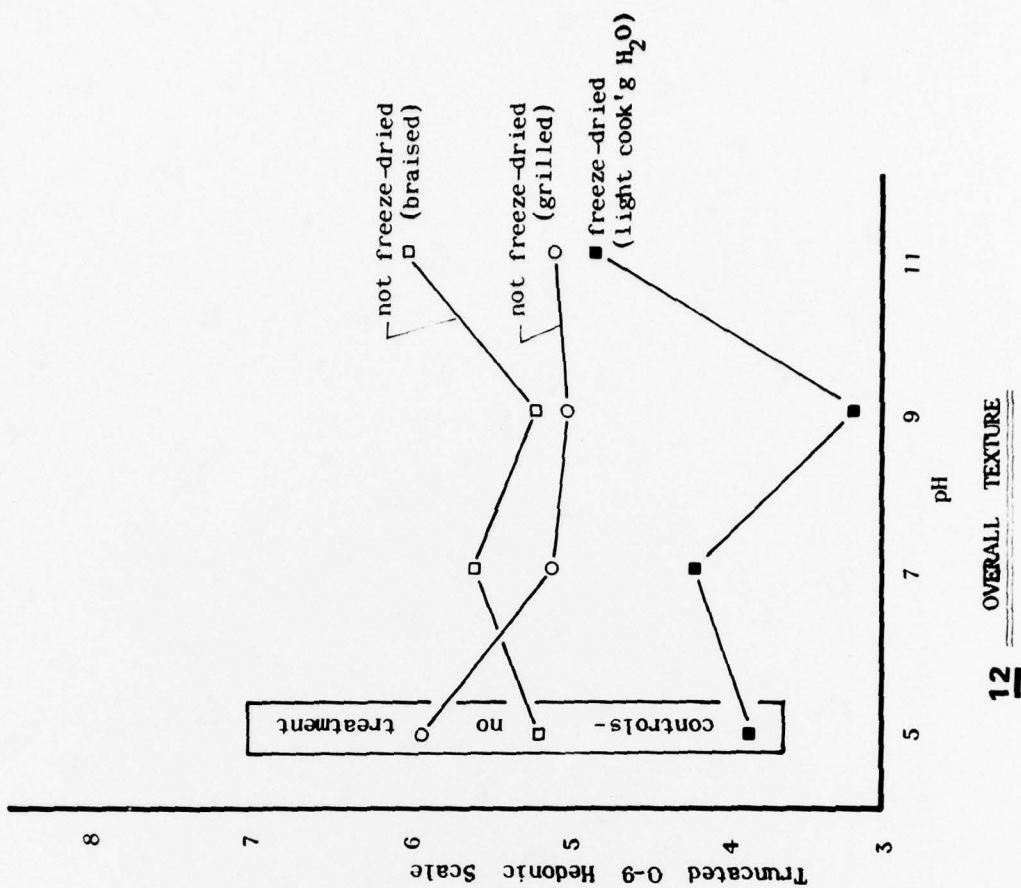
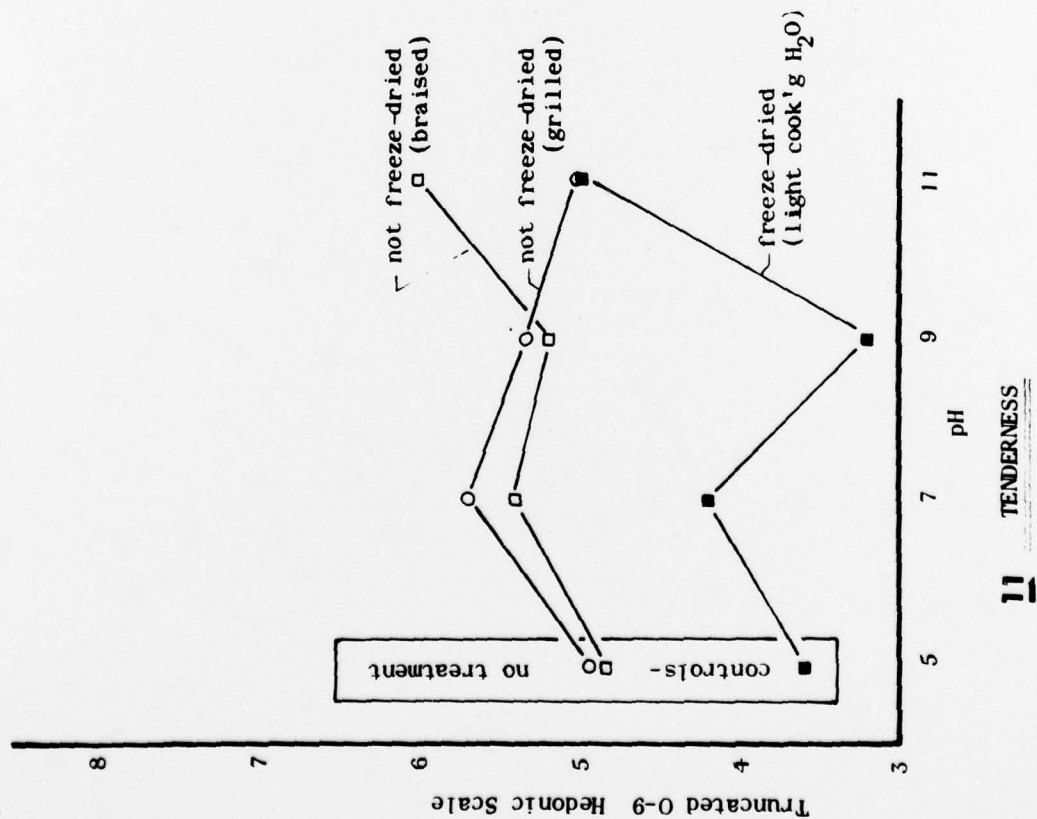
8 COMPARISON OF THE EFFECTS OF pH ON REHYDRATION WEIGHT, AND COOKED WEIGHT OF FREEZE-DRIED AND NON-FREEZE-DRIED MEAT, AND COOKING METHOD.

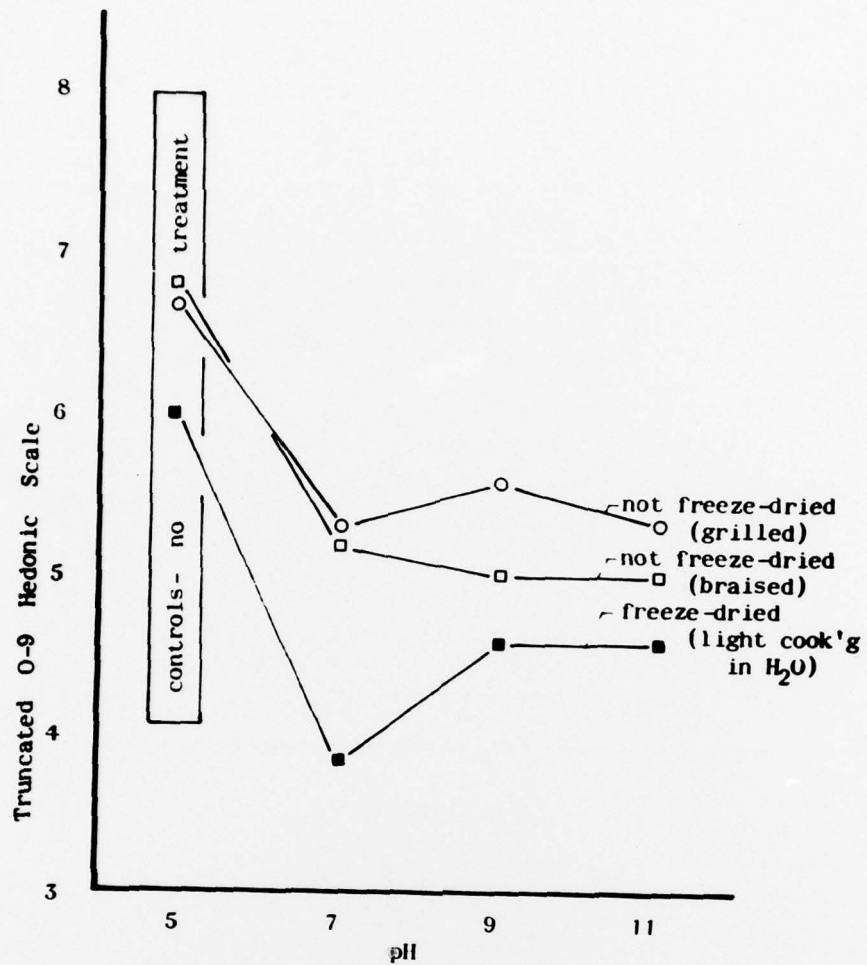


9 EFFECT OF pH VARIATION ON REHYDRATION RATIO



10 JUICINESS





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